FIG. 4.

JDF-3 DNA polymerase genomic sequence (SEQ ID NO: 4)

```
AATTCCACTGCCGTGTTTAACCTTTCCACCGTTGAACTTGAGGGTGATTT
TCTGAGCCTCCTCAATCACTTAATCGAGACCGCGGATTACCTTGAACTGG
TACACGTTCAACGATTCGGTTCTTGTAATGGTCGATACTGGGCCGTGCTG
GATTTTCTAAACGTCTCAAGAACGGCTTTCATCAACGGAAACTGCCACGT   5' untranslated sequence
CTCCGCCGTCGTGAGGGTTAAACCTGAAGTTCAAGACTTTGCAACGGAAT
GGCGAGAGAACGGCGACTACCCCAGTGGAAGAGCTTTTGAAAGCCAAAGC
CGAGCTTCAGCGAATGTGCGGTGCCCTTGTTCAAGAGTTGTGAGCCCTTG
ATTGTTGTTTCTCCTCTTTTCTGATAACATCGATGGCGAAGTTTATTAG
TTCTCAGTTCGATAATCAGGCAGGTGTTGGTC
```

```
                            ATGATCCTTGACGTTGAT
TACATCACCGAGAATGGAAAGCCCGTCATCAGGGTCTTCAAGAAGGAGAA
CGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAGCCCTACTTCTACG
CGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCG
GAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGGTGAAGAA
AAAGTTCCTCGGCAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACC
CGCAGGACGTTCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGCGGTC
ATCGACATCTACGAGTACGACATACCCTTCGCCAAGCGCTACCTCATAGA
CAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCT
TCGACATCGAGACGCTCTACCACGAGGGAGAAGAGTTTGGAACCGGGCCG
ATTCTGATGATAAGCTACGCCGATGAAAGCGAGGCGCGCGTGATAACCTG
GAAGAAGATCGACCTTCCTTACGTTGAGGTTGTCTCCACCGAGAAGGAGA
TGATTAAGCGCTTCTTGAGGGTCGTTAAGGAGAAGGACCCGGACGTGCTG
ATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAGCGCTG
TGAGAAGCTTGGCGTGAGCTTTACCCTCGGGAGGGACGGGAGCGAGCCGA   Extein 1
AGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTA
CACTTCGACCTTTATCCAGTCATAAGGCGCACCATAAACCTCCCGACCTA
CACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCAAGGAGAAGG
TCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGCTTGAG
AGGGTCGCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGG
CAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAG
GCCTCTGGGACGTTTCCCGCTCCAGCACCGGCAACCTCGTCGAGTGGTTC
CTCCTAAGGAAGGCCTACGAGAGGAACGAACTCGCTCCCAACAAGCCCGA
CGAGAGGGAGCTGGCGAGGAGAAGGGGGGGCTACGCCGGTGGCTACGTCA
AGGAGCCGGAGCGGGGACTGTGGGACAATATCGTGTATCTAGACTTTCGT
AGTCTCTACCCTTCAATCATAATCACCCACAACGTCTCGCCAGATACGCT
CAACCGCGAGGGGTGTAGGAGCTACGACGTTGCCCCCGAGGTCGGTCACA
AGTTCTGCAAGGACTTCCCCGGCTTCATTCCGAGCCTGCTCGGAAACCTG
CTGGAGGAAAGGCAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCC
GCTGGAGAAGAATCTCCTCGATTACAGGCAACGCGCCATCAAGATTCTCG
CCAAC
```

```
    AGCCTTCTTCCCGGGGAGTGGGTTGCGGTCATTGAAGGGGGGAAA
CTCAGGCCCGTCCGCATCGGCGAGCTGGTTGATGGACTGATGGAAGCCAG
CGGGGAGAGGGTGAAAAGAGACGGCGACACCGAGGTCCTTGAAGTCGAGG
GGCTTTACGCCTCTCCTTCGACAGGGAGTCCAAGAAAGCCCGCACAATGC
CGGTGAAAGCCGTGATAAGGCACCGCTATGCCGGGGAAGTTTACAGAATA
GCTCTCAACTCCGGAAGGAGGATTAAGCGTGACGCGCGGCCACAGCCTCT   Intein 1
TCGCGTACCGGGACGCGAGCTTGTGGAGGTGACGGGGGAGGAGGAGGTTC
AAGCCCGGCGACCTCCTGGCGGTGCCAAGCGGATAACCCTCCCGGAGAGG
```

AGGGAGAGGCTCAACATCGTTGAACTGCTCCTCGAACTGCCCGAGGAGGA
AACGGCCGACATGTCATCGACATTCCGGCAAGGGTAGAAAGAACTTCTTC
AGGGGAATGCTCAGAACCCTCCGCTGGATTTTCGGGGAGGAGAAGACCGG Intein 1
AGGGCGGCCAGGCGCTACCTGGAGCACCTTGCGTGGGCTCGGCTACGTGA
AGCTGAGGAAAATCGGCTACGGGGTGGTTGATAGGGAGGGACTGGGAAAG
GTACCGCGCTTCTACGAGAGGCTCGTGGAGGTAATCCGCTACAACGGCAA
CAGGGGGGGAGTTCATCGCCGATTTCAACGCGCTCCGCCCCGTCCTCCGCC
TGATGATGCCCGAGAAGGAGCTTGAAGAGTGGCTCGTTGGGACGAGGAAC
GGGTTCAGGATAAGGCCGTTCATAGAGGTTGATTGGAAGTTCGCAAAGCT
CCTCGGCTACTACGTGAGCGAGGGGAGCGCCGGGAAGTGGAAAAAACCGGA
CCGGGGGCTGGAGCTACTCGGTGAGGCTTTACAACGAGGACGGGAGCGTT
CTCGACGACATGGAGAGACTCGCGAGGAGTTCTTTGGGGGCGTGAGCGCG
GGGGGAACTACGTCGAGATTTCAAAGAAGATGGCCTACATAATCTTCGAG
GGGCTCTGCGGTTCACCGGCCGAGAACAAGAGGGGTTCCGTGGCTTATCTT
CACGTCCCCTGAGGAGGTCCGCTGGGCCTTCCTTGAGGGGTACTTCATCG
GCGACGGCGACGTTCACCCGAGCAAGATGGTTCGGCTCTCCACCAAGAGC
GAGCTTCTGGCTAACGGCCTCGTCCTGCTCCTGAACTCGCTGGGCGTCTC
AGCGATAAACGTCCGCCACGACAGCGGGGTTTACAGGGTCTACGTGAACG
AGGAACTGCCCTTTACAGAGTACCGGAAGCGGAAGAACGCCTCACTTACT
CCCACGTCATACCGAGGGAAGTGCTGGAGGAGACTTCGGCCGGGCCTTCC
AGAAGAACATGAGTCACGGGAAATTCAGGGAGCTGGTGGAAAGCGGGGAG
CTCGACGCGGAAAGGGCCGGTAGGATAGGCTGGCTCCTCGACGGGGATAT
AGTCCTCGACAGGGTCTCGGAAGTCAGGAAGGAAAGCTACGAGGGGTACG
TCTACGACCTGAGCGTTGAGGAGGACGAGAACTTCTGGCGGGCTTTGGGT
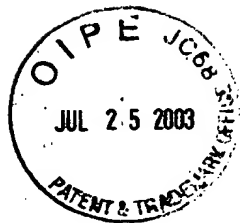TCCTCTACGCGCACAACNN

                    AGCTACTACGGCTACTACGGCTATGCCAGGG
CAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGG
GAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAA
AGTCCTCTATGCAGACACAGACGGTCTCCATGCCACCATTCCTGGAGCGG
ACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAAT
CCCAAACTGCCCGGCCTTCTCGAACTCGAATACGAGGGCTTCTACGTCAG
GGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCA
AGATAACCACGCGCGGGCTTGAGTAGTCAGGCGCGACTGGAGCGAGATA
GCGAAGGAGACGCAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGA Extein 2
CGTTGAAGAGGCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCA
AGTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGC
GAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAGCG
TTTGGCCGCCAGAGGTGTTAAAATCCGGCCCGGAACTGTGATAAGCTACA
TCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGAC
GAGTTCGACCCGACGAAGCACAAGTACGATGCGGACTACTACATCGAGAA
CCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCA
AGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGCGCGTGG
CTGAAGCCGAAGGGGAAGAAGAAGTGA

                    GGAATTATCTGGTTTCTTTTCCC
AGCATTAAATGCTTCCGACATTGCCTTATTTATGAAACTCCTGTTGTGCC
TGAGTTTGTGCCAGAAAACAGCCTGTTCTGACGGCGCTTTTTCTTGCCAG
GTCTCTTGAGTTTCGCAAGGGTCTTCTCGACCAGCTCAATGGTCTTGTCG
TCATTGTTTNNNNNNNNNNNNNNNNNNNNNNNNNCCCGGGGACTTCATACTGGC
GGTAATAGACAGGGATTCCTTCCTCAAGGACTTCCCGGGAGGCATTGGAG
TTTTTTGGTGGGGCTTTCACAGGATTTGCTCATCTTGTGGATTTCTCGTT
CGATTGAATCTGTCCACTTGAGGGTGTAGGTCGAGACGGTGGAGCGCGTA

SUBSTITUTE FIGURE

TTCCGGGAGCGGGTCTTGAGGCTCCATTTTTCAGTCCTCCTCCGGCGAAG 3' Untranslated sequence
AAGTGGAACTCAAGCCGGGTGTTAGCTTATGTTATGTTCCCAACTCCTCC
AGCACCTCCAGGATCCCCTCAATCCCGGAACCTCGAAGCCCCTCTCGTGG
ATCTTTCTAACTTCCTCTGCCTCCGGGTTTATCCAGACCGCCCACATGCC
GGCTCTCAGCGCACCCTCGAAATCCTCCGCGTAGGTGTCGCCGATGTGGA
TTGCCTCGTCCGGCTCGACCCCGAAGCATCGAGCGGTTTTCTGAACATCT
CGGGCATCGGCTTATACGCCAGAACCTCGTCGGCGAAGAAGGTTCCCTCA
ATGTAGTCCATCAGGCCGAACCTCTCGAGGGGGGGCCCGGTACCCAATTC
GCCCTATAGTGAGTCGATTACAATTCACTGGCCGTCGTTTTACAACGTCG
TGACTGGGAAAACCCTGGCGTTACCCAACTTAAGTCGCTTTGCAGCACAT
CCCCC

SUBSTITUTE FIGURE

Preliminary Qualification of Mutants

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | **5A** |

WTXOJDF3

| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | **5B** |

WTXOJDF3

5C

WTXOJDF3
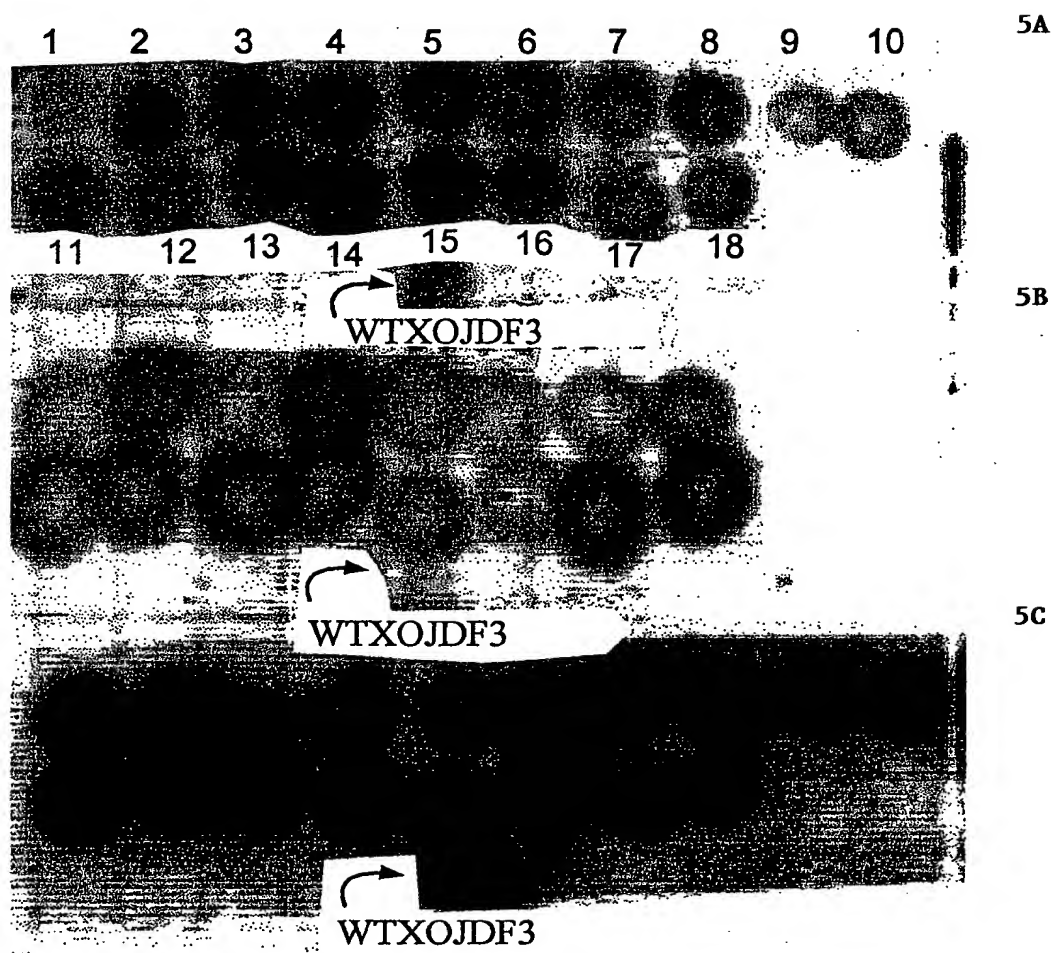
FIG. 5

Sequencing with Purified Mutants
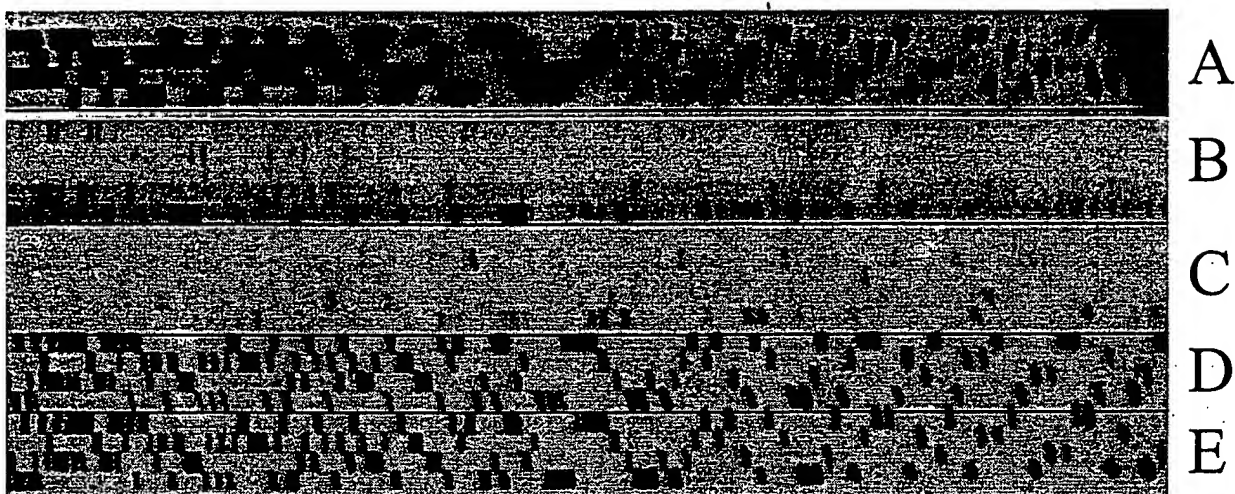
Figure 6

Sequencing with Dye-labeled Dideoxynucleotides

A
B ddA
C
D

A
B ddG
C
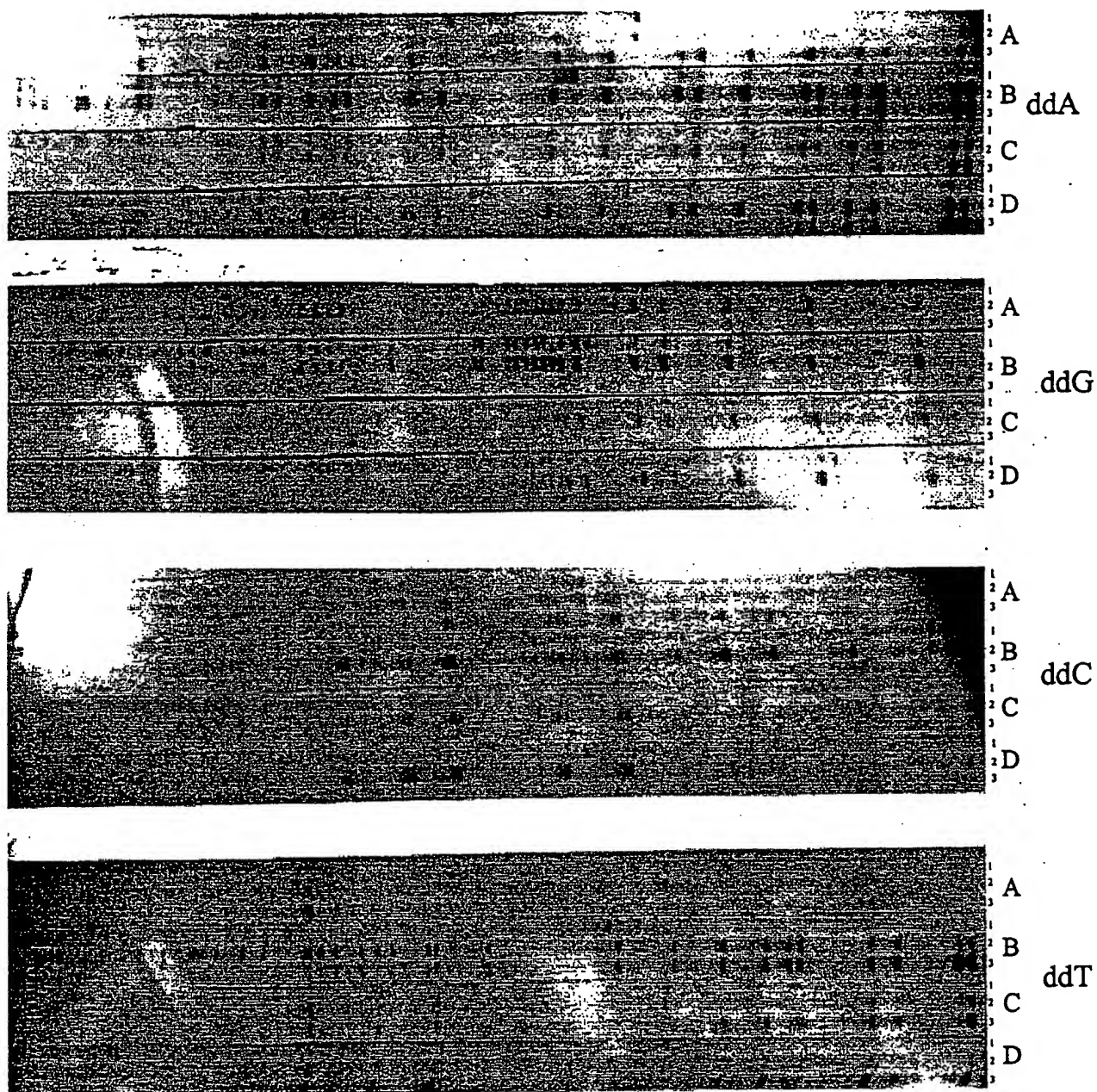D

A
B ddC
C
D

A
B ddT
C
D

Figure 7

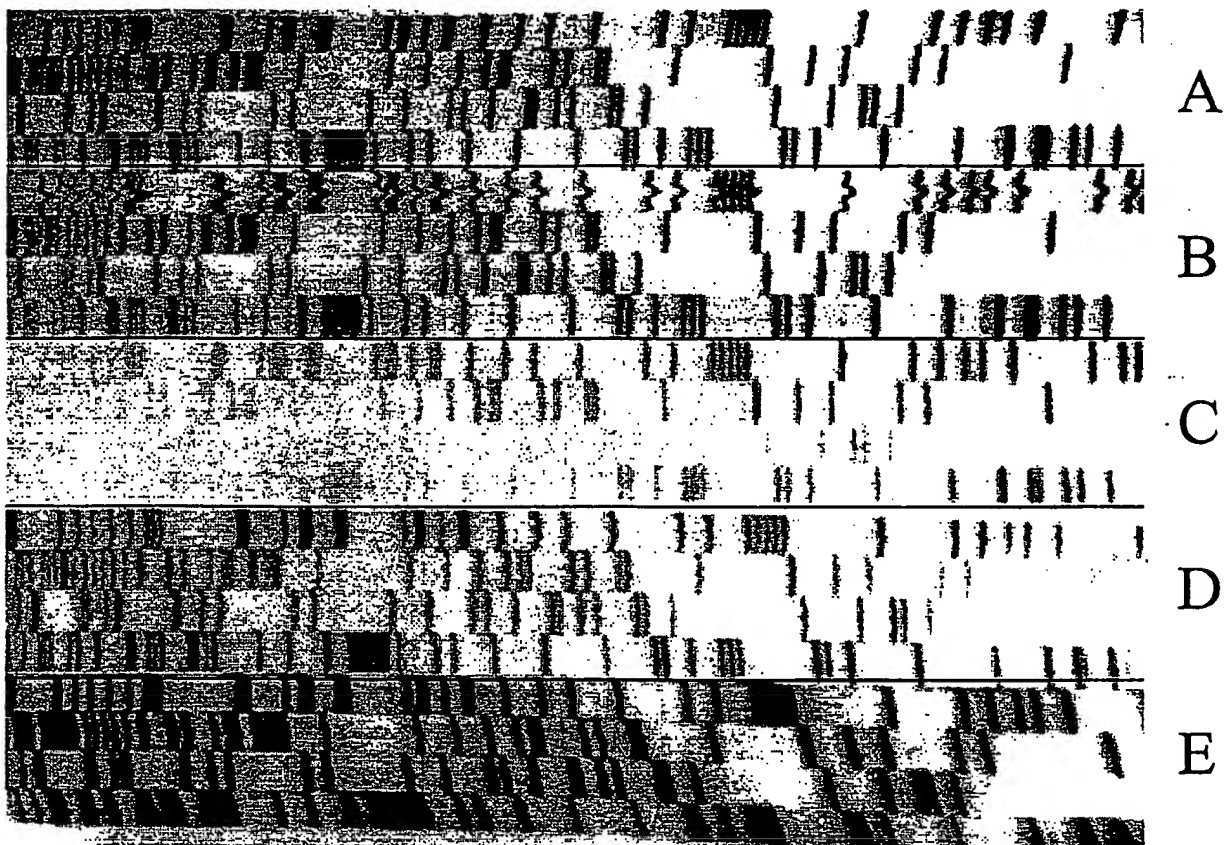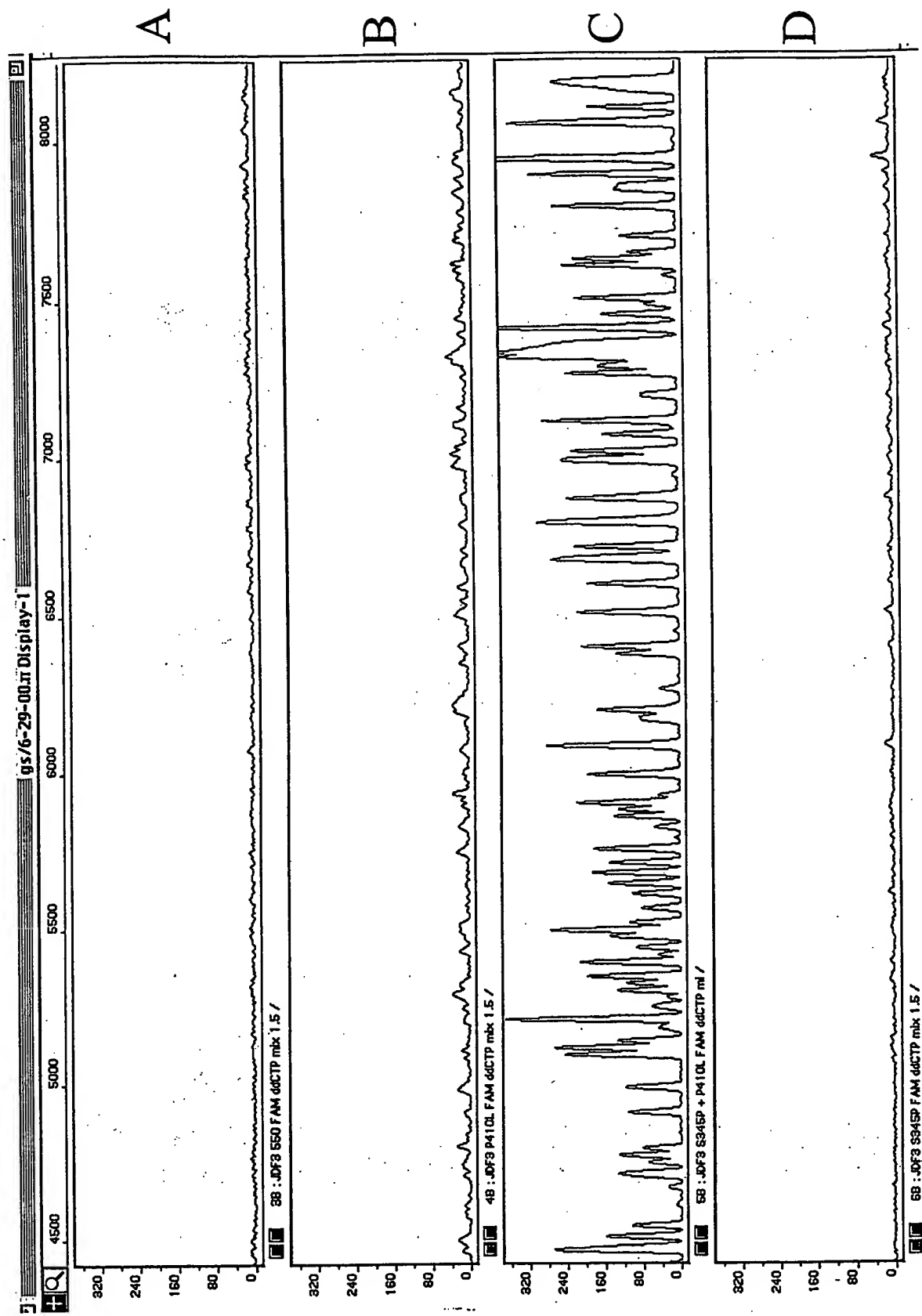## Sequencing with the P410L, A485T Double Mutant and α-³³P Dideoxynucleotides
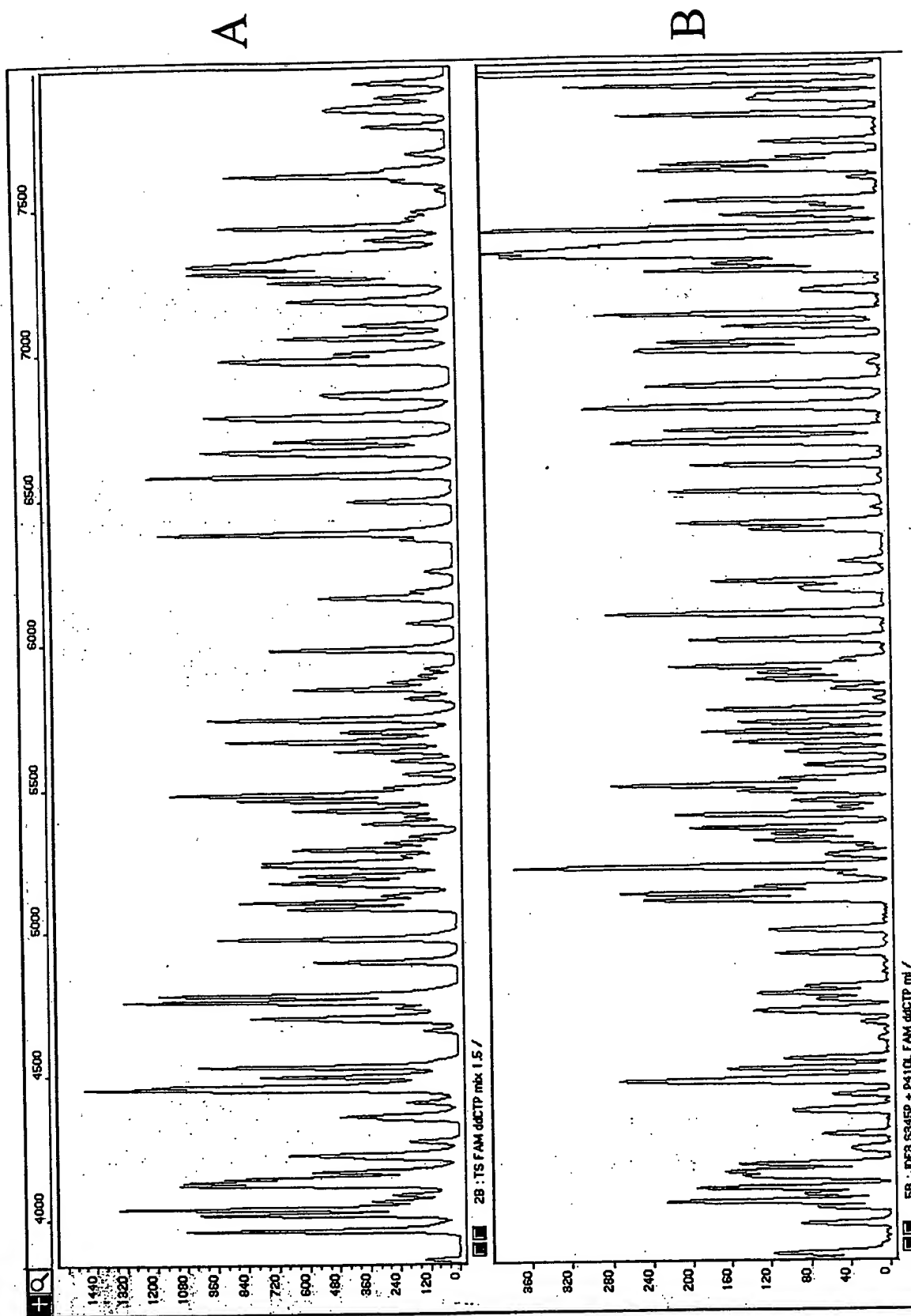

Figure 8

Figure 10

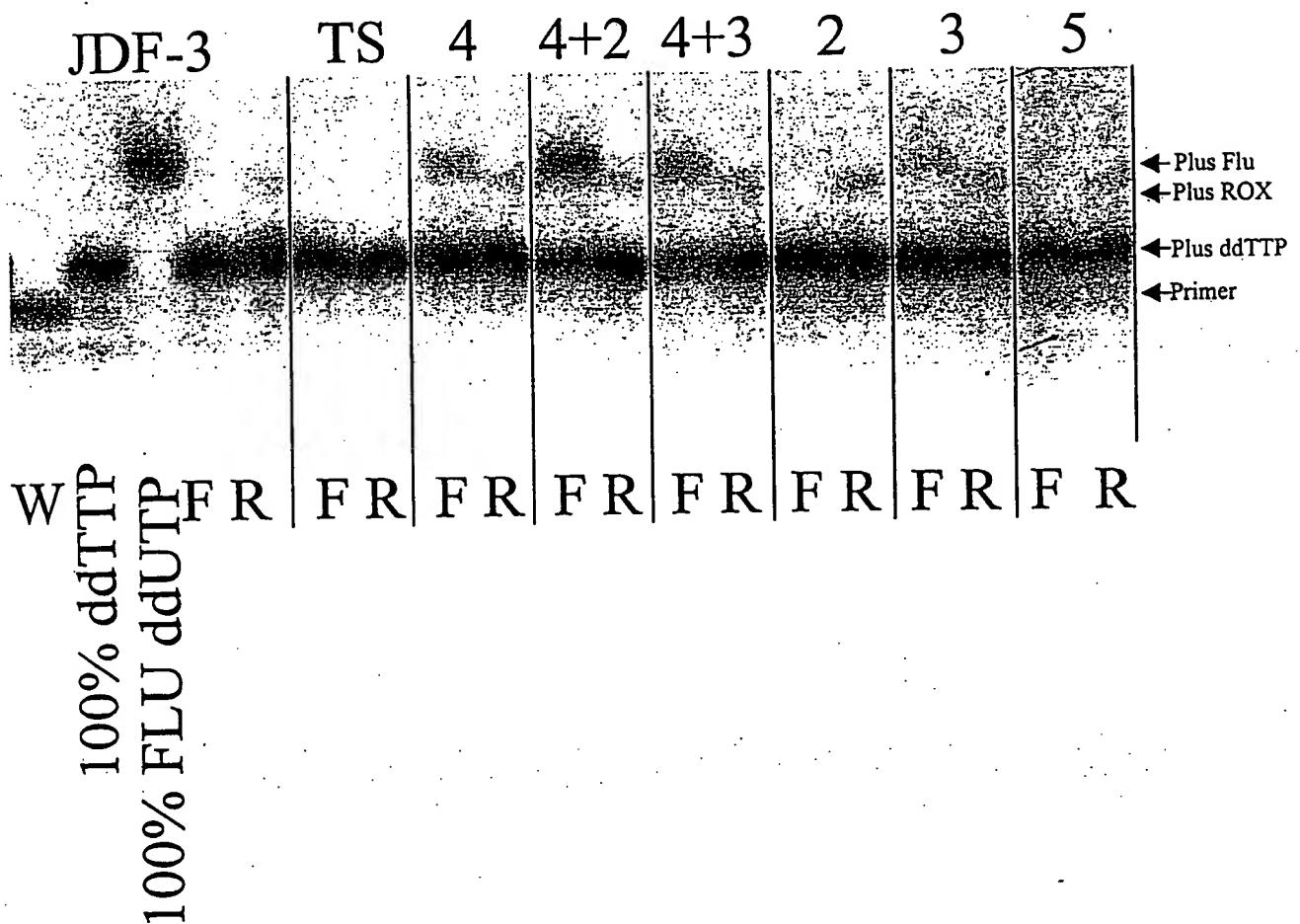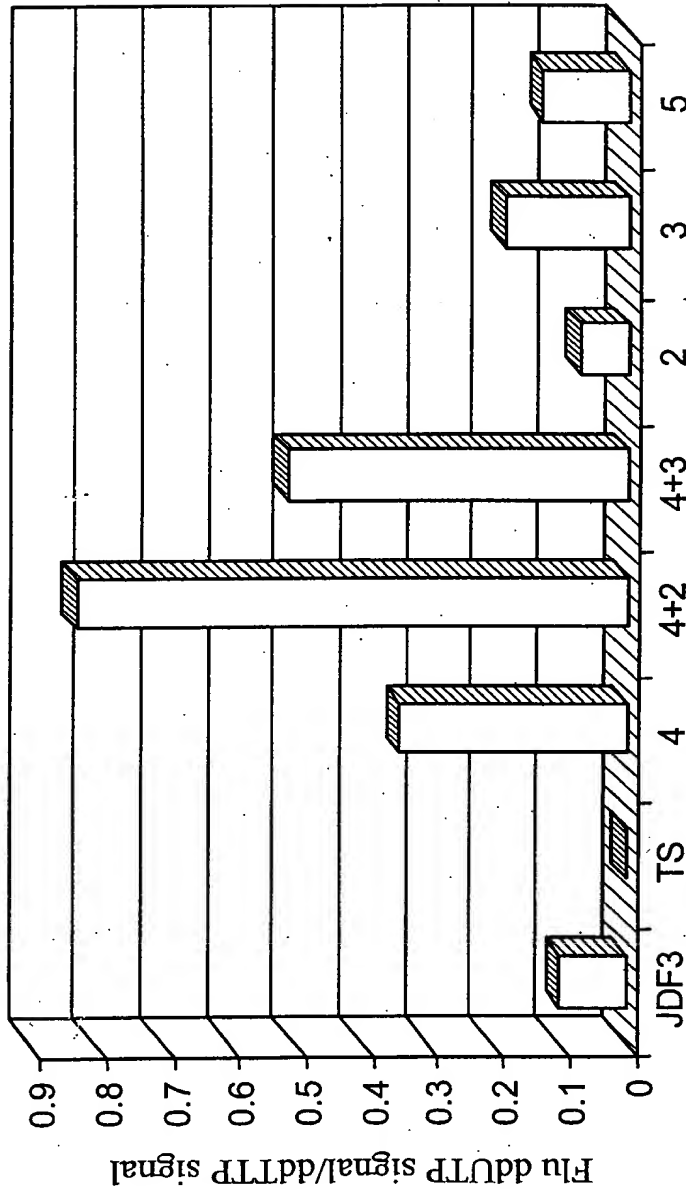Figure 11

^{33}P-TAACGTTGGGGGGGGGGCA→
TGCAACCCCCCCCCGTAT

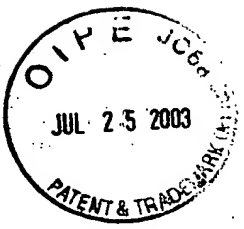JDF-3   TS   4   4+2   4+3   2   3   5

←Plus Flu
←Plus ROX
←Plus ddTTP
←Primer

W FR FR FR FR FR FR FR FR

100% ddTTP
100% FLU ddUTP

Figure 12

FIG. 13

```
4      1   ----------------------------------------LVXNAXSTGNLVEWFLLRK
10     1   ----------------------------------------VWDVSRSSTGNLVERFLLRK
13     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
16     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
18     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
19     1   ----------------------------------------VWDVXRSSTGNLVEWFLLRK
28     1   ----------------------------------------VWDVPRSSTGNLVEWFLLRK
34     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
41     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
33     1   ----------------------------------------VWDVSRSSTGNLVEWFLLRK
48     1   --------------------------------------YWSXPXLRTGNLVEWFLLRK
55     1   -------------------------------------VLGTXPRSSTGNLVEWFLLRK
64     1   ------------------------------------XXXFWDVSRSSTGNLVEWFLLRK
Jdf3   301 TGEGLERVARYSMEDARVTYELGREFFPMEAQLSRLIGQGIWDVSRSSTGNLVEWFLLRK


4      20  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
10     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHSVSP
13     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
16     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
18     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
19     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGQWDNIAYLDFRSLYPSIIITHNVSP
28     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
34     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
41     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGPWDNIVYLDFRSLYPSIIITHNVSP
33     21  AYERNKLAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
48     21  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
55     22  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSHYPSIIITHNVSP
64     24  AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP
Jdf3   361 AYERNELAPNKPDERELARRRGGYAGGYVKEPERGLWDNIVYLDFRSLYPSIIITHNVSP


4      80  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
10     81  DTLDREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
13     81  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
16     81  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKMKMKATLDPLEKNLLD
19     81  DTLKREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
28     81  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
34     81  DTLNREGCRSYXVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
41     81  DTLNREGCRSYXVAPEVGHKFCKDFPGFIPSLLGNLLEVRQKIKRKMKATLDPLEKNLLD
33     81  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
48     81  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNPLEERQKIKRKMKATLDPLEKNLLD
55     82  DTLNREGCRSYDVAPEDGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNHLD
64     84  DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
Jdf3   421 DTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMKATLDPLEKNLLD
```

Figure 14

SUBSTITUTE FIGURE

```
  4   140  YRQRAIKILANSYYGYCGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 10   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 13   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 16   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 18   141  YRQRAIKILANNYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 19   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 28   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 34   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 41   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 33   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 48   141  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 55   142  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
 64   144  YRQRAIKILANSYYGNYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD
Jdf3  481  YRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYAD


  4   200  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 10   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 13   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 16   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELKYEGFYVRGFFVTKKKYAVIDEE
 18   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 19   201  TDGLHATIPGADAETVKKKAMEFLNYINLKLPGLLELEYEGFYVRGFFVTKKKXAVIDEE
 28   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 34   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 41   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 33   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLEPEYEGFYVRGFFVTKKKYAVIDEE
 48   201  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 55   202  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
 64   204  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE
Jdf3  541  TDGLHATIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEE


  4   260  GKITTRGLEIVRRDWSEIAKETQARVLEAVLRHGDVEEAVRIVREVTEKLSKYEVPPEKL
 10   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEEL
 13   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVRKVTEKLSKYEVPPEKL
 16   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
 18   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHDDVEEAVRIVREVTEKLSKYEVPPEKL
 19   261  GKITTRGLEIVRRDWSKIAKETQARVLEAILRHGDVEEAIRIVREVTEKLSKYEVPPEKL
 28   261  GKIATRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
 34   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLNKYEVPPEKL
 41   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
 33   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
 48   261  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPVKL
 55   262  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPGEA
 64   264  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
Jdf3  601  GKITTRGLEIVRRDWSEIAKETQARVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKL
```

Figure 15

SUBSTITUTE FIGURE

Preliminary Qualification of Mutants

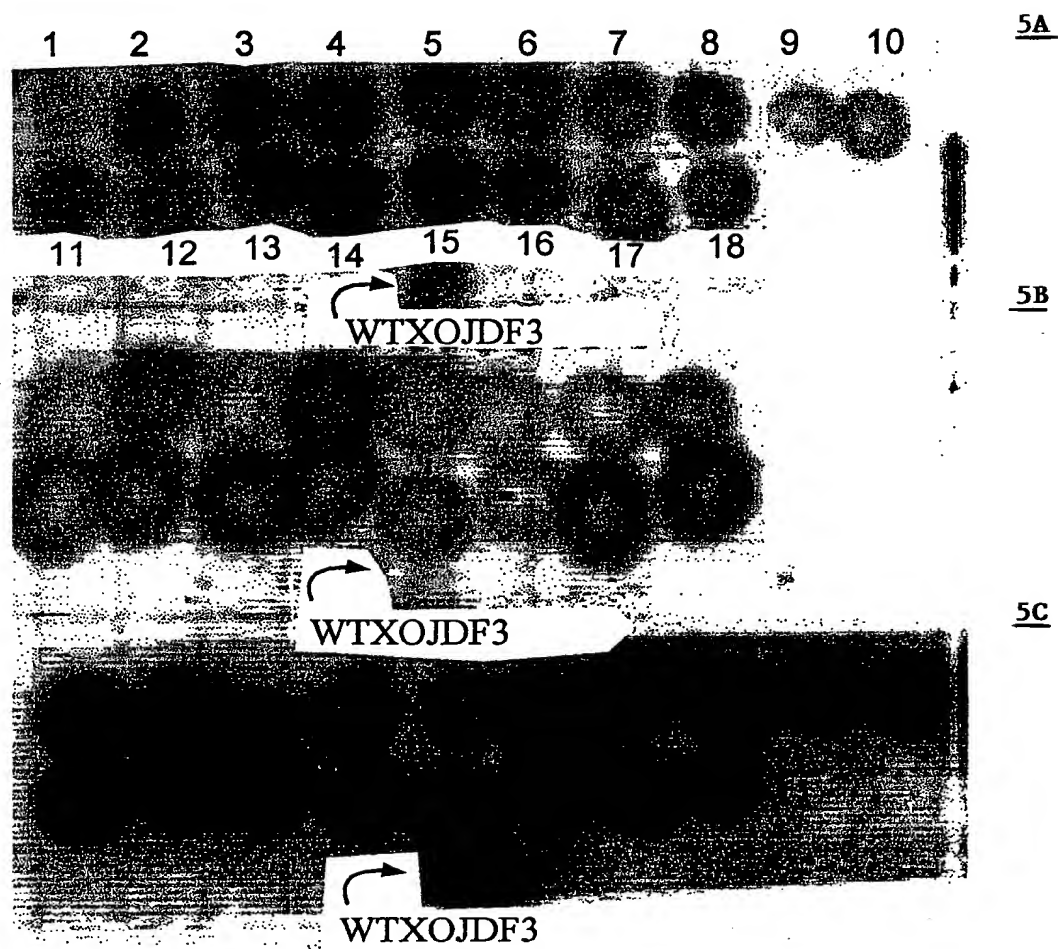FIG. 5